# THE EFFECT OF CERTAIN NARCOTICS (URETHANES) ON PERMEABILITY OF LIVING CELLS TO WATER

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The experiments on narcotics reported in this paper were carried out with the purpose of gaining further information on the factors which regulate or influence permeability of the living cell to water. Former studies on sea urchin eggs have shown that cell permeability to water is not constant, but varies with a number of factors, an important one being the chemical composition of the medium (McCutcheon and Lucké, 1928). Thus permeability is regulated, at least in part, by the sign and the number of charges on the ions of the medium, in the sense that anions increase and cations decrease permeability to water; the effects are the greater the higher the valence of the ions (Lucké and McCutcheon, 1929). In the presence of cations of two or more valences permeability is of very low magnitude. For example, in hypotonic sea water cells, such as unfertilized eggs of the sea urchin, have a numerical value of permeability of approximately 0.05 at 15° C., *i.e.*, 0.05 cubic micron of water pass through each square micron of cell surface per minute, under the driving force of one atmosphere of osmotic pressure.[1] This surprisingly low degree of permeability is presumably due to the high concentration of calcium and magnesium in sea water, for the same low value of permeability is obtained when cells are placed in a non-electrolyte medium, of like osmotic pressure, containing as little as 0.0001 molar $CaCl_2$ or $MgCl_2$. But all attempts to further decrease permeability have so far been unsuccessful. However, several writers have reported that narcotics decrease permeability to water (Winterstein, 1916; Lillie, 1918; and Anselmino, 1928). The question therefore arose whether narcotics in the presence of sea water, or narcotics in any solution containing cations of two or more valences, would lower permeability beyond the value obtained in sea water alone.

## Method

A satisfactory method of studying quantitatively the effect of various factors on permeability to water is to place a suitable cell in a

[1] By permeability to water is understood the quantity of water passing through unit area of cell surface in unit time under unit pressure.

hypotonic medium, thus causing water to enter the cell under the driving force of osmotic pressure. The spherical unfertilized egg of the sea urchin, *Arbacia punctulata*, is an excellent natural osmometer (McCutcheon and Lucké, 1926; McCutcheon, Lucké and Hartline, 1931 *a* and *b*). When placed in a hypotonic solution it swells relatively slowly, thus permitting accurate measurement of its diameter, from which volume and surface area can be calculated; it has a high degree of semipermeability, retains its spherical shape during change of volume, and allows ready determination of injury which may have taken place during the experiment.

The narcotics selected for this study are certain urethanes and carbamates.[2] These compounds have the advantage of being non-injurious over a considerable range of concentration; they are non-volatile, penetrate almost instantaneously, and their narcotic effect is easily demonstrable with the material used.[3]

In the first series of experiments the narcotics were dissolved in ordinary (100 per cent) sea water. Unfertilized eggs of *Arbacia* were exposed to these solutions for from 5 to 10 minutes. The cells were then transferred to hypotonic sea water (usually 40 per cent sea water) with the narcotic to be tested in the same concentration as in the isotonic solution. The course of inflow of water at constant temperature ($15 \pm 0.5°$ C.) was then observed by measuring the diameter of three cells at minute intervals with a filar micrometer for six successive minutes.[4] The mean volume of the cells was plotted against time and a smooth curve drawn through the points. The rate of passage of water is given by the rate of change of volume, $dV/dt$, and is obtained from the slope of the curve at a given time, $t$. The numerical value of permeability to water is calculated from the equation:

$$\text{Permeability} = \frac{dV}{dt} / S(P - P_{ex}),$$

where $S$ is the surface area of the cell and $(P - P_{ex})$ the difference in pressure between the interior of the cell at time $t$ and the medium.[5]

[2] For the sake of brevity the compounds will be referred to in this paper as urethanes. The propyl urethanes were not commercially obtainable, and were kindly supplied by Dr. Ralph Major, then of Princeton University.

[3] The narcotic effect of these compounds will be dealt with by Dr. E. B. Harvey in a forthcoming paper.

[4] The technic of measuring volume changes in *Arbacia* eggs by means of a filar micrometer eye-piece has been previously described (McCutcheon and Lucké, 1926).

[5] For details of calculation see McCutcheon and Lucké, 1928, 1929, and Lucké, Hartline and McCutcheon, 1931*b*.

### Effect of Urethanes When Dissolved in Sea Water

Before determining the effect of these narcotics on permeability a preliminary question needed to be answered: Do these compounds affect the volume of cells in equilibrium with either an isotonic or hypotonic medium? If they should not enter practically instantaneously the volume of the cells would decrease when transferred to isotonic sea water containing a considerable concentration of the narcotic (*e.g.*, 0.2 m. ethyl urethane). On the other hand, if these compounds do enter rapidly, but cause severe injury or death, the volume of the cell in equilibrium with a hypotonic solution might be either too small (from escape of dissolved substances) (Lucké and McCutcheon, 1930) or too large (from splitting of substances in the interior of the cell) (Lucké and McCutcheon, 1926). Now, in order to use the simple equation for permeability given above it is necessary that the equilibrium volume of the cells in the solution to be tested should be the same as the volume at equilibrium of control (unnarcotized) cells in both isotonic and hypotonic concentrations of their natural medium, sea water. This question was answered as follows:

Different lots of cells from the same animal were placed in sea water in which had been dissolved a narcotic in the concentrations shown in Table I. After 5 to 10 minutes' exposure a number of cells were measured and then transferred to a hypotonic solution containing the same concentration of the urethanes. The volume at equilibrium was

Table I

*Effect of Urethanes on Cell Volume at Equilibrium*

The concentrations shown are the highest used in the experiments. $V_o$ is the mean volume of 25 cells measured after from 5 to 10 minutes' exposure to the narcotic solution in isotonic (100 per cent) sea water, $V_e$ is the mean volume of 25 cells in equilibrium with a given hypotonic solution in which were dissolved the different urethanes (measured 4 hours after transfer). It is seen that the volume of cells in the narcotic solutions corresponds closely with the volume of control cells in the same concentration of sea water. The figures must be multiplied by 100 to obtain volumes in cubic micra.

| Solution | $V_o$ | $V_e$ |
|---|---|---|
| First sea water control | 1908 | 3255 |
| Second sea water control | 1882 | 3230 |
| Ethyl urethane 0.2 m | 1933 | 3182 |
| n-propyl urethane 0.1 m | 1920 | 3230 |
| i-propyl urethane 0.1 m | 1876 | 3182 |
| n-butyl carbamate 0.05 m | 1884 | 3275 |
| Phenyl urethane 0.002 m | 1924 | 3273 |

determined after 4 hours' exposure at 22° C. Table I shows the result of a representative experiment. It is seen that there is no change in volume of the narcotized cells in isotonic sea water, and the final equilibrium attained in hypotonic solutions corresponds to that of the control (unnarcotized) cells.[6] Other experiments gave similar results.

It was, therefore, possible to study the effect of these narcotics on cellular permeability to water by the method outlined above. In Table II are given three experiments, representative of a larger number. The table shows that none of the urethanes over a considerable range of definitely narcotic concentration caused significant change in permeability from the controls, excepting that increase in permeability occurred when the cells became injured during the experiment.[7]

These and similar experiments lead to the conclusion that urethanes in narcotic concentration when dissolved in sea water do not decrease permeability of the living cell to water.

## Effect of Urethanes When Dissolved in a Non-Electrolyte Medium

The result obtained with narcotics when dissolved in sea water, *i.e.*, failure to decrease cell permeability to water, may possibly be explained on the grounds that at a given temperature permeability can be reduced only to a certain value, and that this value is the one normally obtained in sea water. Since, then, the presence of the bivalent cations of sea water might, perhaps, mask the action of narcotics, the experiments were repeated in hypotonic dextrose solution.

The experiments were carried out as follows: Unfertilized eggs of *Arbacia* were washed in 0.95 molal solution of dextrose, isotonic with sea water, to eliminate electrolytes from the medium. The cells were then caused to swell in 0.38 molal dextrose solution, isotonic with 40 per cent sea water. Other eggs from the same animal were washed in isotonic dextrose solutions containing in the one case urethane, in the other calcium chloride; they were then measured during the course of swelling in hypotonic dextrose solution to which had been added ure-

[6] It should be pointed out that *prolonged* exposure to urethane solutions may change the shape of cells. This is especially the case in the more concentrated solutions of ethyl and propyl urethane, in which the normally spherical cells may become transformed to bizarre amoeboid forms. No such changes occurred when cells were exposed to less concentrated solutions, for a shorter length of time and at a lower temperature. In the experiments here reported, cells retained their normal shape.

[7] The narcotics, in the experiments here reported, were used in three different concentrations, of which the lowest was still definitely narcotizing and the highest not toxic under the conditions of the experiment. Still higher concentrations proved injurious and increased permeability.

The narcotizing effect was kindly determined by Dr. E. B. Harvey on the basis of cleavage experiments.

## TABLE II

*Urethanes in Sea Water*

In these three experiments the narcotics in the molar concentrations shown are dissolved in 40 per cent sea water (40 parts of sea water and 60 parts of distilled water). In the last column of the table is given the value of permeability, which is the number of cubic micra of water entering the cell per minute, per square micron of surface, per atmosphere of pressure. The temperature was 15 ± 0.5° C.

It is seen that the various urethanes do not decrease permeability beyond the value obtained in sea water alone. The increased values of permeability (indicated by an asterisk) were obtained in injured cells, *i.e.*, in cells which failed to cleave when returned to ordinary sea water and inseminated at the conclusion of the experiment.[8]

| Compound | Concentration | Permeability |
|---|---|---|
| Ethyl urethane | 0.2 | 0.055 |
| " " | 0.1 | 0.057 |
| " " | 0.05 | 0.056 |
| First sea water control | | 0.054 |
| Second sea water control | | 0.054 |
| n-propyl urethane | 0.1 | 0.096* |
| n-propyl urethane | 0.05 | 0.052 |
| n-propyl urethane | 0.025 | 0.056 |
| i-propyl urethane | 0.1 | 0.057 |
| i-propyl urethane | 0.05 | 0.051 |
| i-propyl urethane | 0.025 | 0.055 |
| First sea water control | | 0.058 |
| Second sea water control | | 0.053 |
| n-butyl carbamate | 0.05 | 0.089* |
| n-butyl carbamate | 0.025 | 0.061 |
| n-butyl carbamate | 0.0125 | 0.063 |
| i-amyl carbamate | 0.01 | 0.059 |
| i-amyl carbamate | 0.005 | 0.061 |
| phenyl urethane | 0.005 | 0.060 |
| phenyl urethane | 0.0025 | 0.057 |
| phenyl urethane | 0.00125 | 0.056 |
| First sea water control | | 0.059 |
| Second sea water control | | 0.069 |

[8] It has previously been shown (Lucké and McCutcheon, 1930) that injury induced by high temperature and by anisotonic solution causes an increase in cellular permeability to water. From the experiments given in the table as well as from similar experiments, it is evident that injury induced by toxic concentrations of narcotics also increases permeability to water. Higher concentration of narcotics than those employed produced injury, and hence increased permeability.

thane or calcium, in the same concentration as was present in the isotonic solution of dextrose. Permeability was therefore determined, in each experiment, in pure dextrose solution, in dextrose solution containing a narcotic, and in dextrose containing calcium.

The results of three such experiments are shown in Table III. It is seen that the values of permeability in pure dextrose solution are about twice as great as in calcium-dextrose solution, while permeability values

TABLE III

*Urethanes in Dextrose*

The narcotics in the molar concentrations shown are dissolved in 0.38 molal solution of dextrose. In the top row is given the permeability of cells in pure dextrose solution and in the bottom row the permeability in dextrose solution containing 0.01 m. $CaCl_2$. Each permeability value in this experiment is based on measurements of ten cells. The temperature was 12° ± 0.5° C.

It is seen that the narcotics cause a definite decrease in permeability which, however, is not as great as the decrease effected by calcium.

| Solution | Permeability | | |
|---|---|---|---|
| Dextrose 0.38 m | 0.096 | 0.097 | 0.092 |
| Dextrose 0.38 + n-butyl carbamate 0.025 m | 0.062 | | |
| Dextrose 0.38 + i-amyl carbamate 0.01 m | | 0.085 | |
| Dextrose 0.38 + phenyl urethane 0.0025 m | | | 0.070 |
| Dextrose 0.38 + $CaCl_2$ 0.01 m | 0.041 | 0.047 | 0.041 |

of the narcotized cells lie about midway. A number of similar experiments gave the same results. In every case exposure to the narcotic caused a definite decrease in permeability, which was never of the magnitude of the decrease effected by calcium. The conclusion may be drawn that narcotics tend to decrease cell permeability to water but that their effect may be masked by the presence of cations in the medium.

## DISCUSSION

Most studies of the effect of narcotics on cell permeability have been concerned only with permeability to various substances in solution.[9] The most important investigations on permeability to water, as influenced by narcotics, are those of Winterstein (1916), Lillie (1918), Heilbrunn (1925) and Anselmino (1928).

Winterstein in his first group of experiments used sartorius muscle of frogs, employing the usual method of weighing. He found that in hypotonic solution of sodium chloride containing alcohol in narcotic concentration the weight increase of muscle was less than in the same

[9] The literature on this subject is reviewed by Gellhorn, E., Das Permeabilitätsproblem, Berlin, 1929; Winterstein, H., Die Narkose, Berlin 1926; Lillie, R. S., Protoplasmic Action and Nervous Action, Chicago, 1924; Bayliss, W. M., Principles of General Physiology, 4th edition, London, 1924; Osterhout, W. J. V., Injury, Recovery, and Death in Relation to Conductivity and Permeability, Philadelphia and London, 1922; Jacobs, M. H., in Cowdry, E. V., General Cytology, Chicago, 1924; Höber, R., Physikalische Chemie der Zelle und der Gewebe, Leipsic, 6th edition, 1926; von Tschermak, A., Allgemeine Physiologie, I, Berlin, 1924.

hypotonic solution containing no narcotic. In later experiments Winterstein constructed artificial "cells"; glass cylinders were covered with the thin abdominal muscle of female frogs. It was found that, in agreement with the experiments on sartorius muscle, four different narcotics effected a marked decrease of the water intake by the "cell." The effects were reversible.

Lillie investigated the effect of narcotics on permeability to water of fertilized eggs of *Arbacia*. He had previously shown that in the *Arbacia* egg fertilization is followed by an approximately four-fold increase in permeability to water. This increase of permeability was found to be inhibited by various organic anesthetics. In all cases eggs which were caused to shrink, two or three minutes after insemination, in solutions of these compounds in sea water of the appropriate concentrations, remained in the condition of low permeability characteristic of the unfertilized egg. This effect of narcotics was readily reversible.

Heilbrunn repeated Winterstein's experiments, studying the increase in weight of frog's gastrocnemius muscle in distilled water, and in distilled water to which two per cent by volume of ether had been added. He found that water entered etherized muscle somewhat less rapidly than normal muscle. In further experiments Heilbrunn investigated the rapidity of swelling of unfertilized eggs of *Arbacia* in hypotonic sea water containing one or two per cent of ether. His curves show that the cells swelled even more readily in the presence of ether than in its absence. From these experiments Heilbrunn concluded that ether does not lower the permeability of sea urchin eggs to water.[10]

Very recently, Anselmino investigated the effect of various narcotics on permeability to water of dried collodion and of copper ferrocyanide membranes, and found, in agreement with the work of Winterstein on living membrane, a marked decrease of permeability to water.

From the experiments summarized above (excepting Heilbrunn's experiment on *Arbacia* eggs) the conclusion was drawn that narcotics decrease permeability to water. It would appear, however, that the chemical composition of the medium is a factor of importance when investigating the action of narcotics on cell permeability. The experiments reported in the present paper indicate that narcotics, at least in the case of the unfertilized egg of *Arbacia*, do not lower permeability to water when they are caused to act in sea water, or in a medium containing calcium.

[10] It is not improbable that the more rapid swelling of the narcotized cells in Heilbrunn's experiments is due to injury.

SUMMARY

1. The effect of narcotics (urethanes and carbamates) on cell permeability to water was studied by measuring the rate of swelling of unfertilized eggs of the sea urchin, *Arbacia punctulata*, in hypotonic sea water and in hypotonic dextrose solution.

2. Narcotics in the presence of sea water do not decrease permeability to water beyond the value normally found in sea water.

3. But narcotics have a tendency to reduce permeability to water, being, however, less effective in this respect than are bivalent cations. This tendency to decrease permeability is demonstrated when narcotics are used in solutions free from bivalent cations, *i.e.*, in hypotonic solutions of dextrose.

4. The effect of narcotics on permeability to water depends on the chemical composition of the medium in which the narcotizing compound is dissolved.

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